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Tissue-specific expression of the R_I and R_{II} sodium channel subtypes

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ABSTRACT **Anti-peptide antibodies that distinguish between the rat brain sodium channel subtypes referred to as R_I and R_{II} were prepared and used to determine their relative expression in nerve and muscle tissues. Sodium channels purified from rat brain are approximately 18% R_I and 80% R_{II}. In brain, the R_{II} subtype is preferentially expressed with R_I/R_{II} ratios ranging from 0.07 in the hippocampus to 0.17 in the cerebral cortex. The R_I subtype is preferentially expressed in more caudal areas of the central nervous system with values of R_I/R_{II} of 0.98 for medulla oblongata and 2.2 for spinal cord. Expression of additional unidentified sodium channel subtype(s) is detected in midbrain, medulla, and spinal cord, and expression of unidentified sodium channel subtypes predominates over expression of R_I and R_{II} in retina and optic nerve. The R_I and R_{II} subtypes are primarily expressed in the central nervous system and are not detected in significant numbers in skeletal or cardiac muscle, sympathetic ganglia, adrenal medulla, sciatic nerve, or cauda equina. The R_{II} subtype appears first in development of both brain and spinal cord but declines in adult spinal cord as the R_I subtype increases. The strict regional expression of these two sodium channel subtypes suggests that they may have distinct functional properties or physiological roles.**

Sodium channels isolated in functional form from rat brain, rat and rabbit skeletal muscle, and electric eel electroplax all contain a large glycoprotein subunit of 260 kDa as their principal component (reviewed in refs. 1–4). In brain and skeletal muscle, this α subunit is associated with one or two smaller β subunits (3, 4). cDNA clones encoding the primary structure of the α subunits from electroplax (5) and rat brain (6–9) have been isolated, and the complete primary structures of α subunits from electroplax and rat brain have been determined (5, 8). High molecular weight mRNA from rat brain (10), α-subunit mRNA isolated by hybrid selection (9), and α-subunit mRNA synthesized from cloned cDNA (11) all direct the synthesis of functional sodium channels in *Xenopus* oocytes, indicating that the α-subunit mRNA contains all the information necessary to specify a functional sodium channel in this expression system.

Two recent lines of investigation indicate that there are multiple subtypes of sodium channels expressed in mammalian neurons. Polyclonal antibodies directed against the α subunit of the rat brain sodium channel do not recognize sodium channels in peripheral neurons (12). Moreover, cDNA clones encoding three different α-subunit mRNAs have been detected in rat brain, and two of these have been fully sequenced (8). These two mRNAs encode α-subunit subtypes called R_I and R_{II} (8), which have 87% identity in their predicted amino acid sequences. In these studies, we have analyzed the tissue-specific expression of the R_I and R_{II} sodium channel subtypes using sequence-directed antibodies that distinguish these two forms.

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EXPERIMENTAL PROCEDURES

Materials. The catalytic subunit of cAMP-dependent protein kinase (13), saxitoxin (14, 15), purified sodium channels (16), and ^{32}P-labeled sodium channels (17) were prepared as described.

Synthetic Peptides. Peptides SP1 (Cys-Ala-Tyr-Glu-Glu-Gln-Asn-Gln-Ala-Thr-Leu-Glu-Glu-Ala-Glu-Asn-Lys-Glu-Ala), corresponding to residues 425–442 of R_I (8) or residues 427–444 of R_{II} (8) plus an N-terminal cysteine extension, $SP11_I$ (Lys-Thr-Ala-Ser-Glu-His-Ser-Arg-Glu-Pro-Ser-Ala-Ala-Gly-Arg-Leu-Ser-Asp), corresponding to residues 465–481 of R_I (8) plus an N-terminal lysine extension, and $SP11_{II}$ (Lys-Ala-Ser-Ala-Glu-Ser-Arg-Asp-Phe-Ser-Gly-Ala-Gly-Gly-Ile-Gly-Val-Phe-Ser-Glu), corresponding to residues 465–484 (8) plus an N-terminal lysine extension were synthesized by the solid-phase method (18) and purified by reversed-phase HPLC on a Vydac 218TP10 (10-μm particle size) column. The identity of the purified peptides was verified by amino acid analysis and determination of the amino acid sequence. SP1 was radiolabeled with ^{125}I by the chloramine T method (19); $SP11_I$ and $SP11_{II}$ were radiolabeled by reaction with the Bolton–Hunter reagent (20).

Preparation of Antibodies. The purified peptides were coupled through amino groups to bovine serum albumin with glutaraldehyde (21), dialyzed against phosphate-buffered saline, emulsified in an equal volume of Freund's complete (initial injection) or incomplete adjuvant, and injected in multiple subcutaneous sites on New Zealand White rabbits at 3-week intervals. Antisera were collected after the second injection and tested by radioimmunoassay (17). Antibodies were purified by antigen affinity chromatography (12, 22).

Preparation of Tissue Fractions. A crude synaptosomal membrane fraction (P_3) was prepared from whole rat brain as described (23). Electric eel brain and regions of rat brain were homogenized in sucrose buffer (3.3 ml per gram of tissue) consisting of 5 mM EDTA, 5 mM EGTA, 300 mM sucrose (pH 7.4), phenylmethylsulfonyl fluoride (50 μg/ml), 1 μM pepstatin A, and 1 mM iodoacetamide. Debris was removed by centrifugation at 800 × *g* for 10 min, and the membranes were collected by centrifugation at 100,000 × *g* for 60 min. Brains from monkey, chicken, gecko, and frog were homogenized in 320 mM sucrose/5 mM potassium phosphate, pH 7.4/1.5 μM phenylmethylsulfonyl fluoride/1 μM pepstatin A/1 mM iodoacetamide. Freshly dissected retinae and optic nerves were homogenized in 130 mM choline chloride/5.4 mM KCl/0.8 mM $MgCl_2$/5.5 mM glucose/50 mM Hepes (adjusted to pH 7.4 with Tris)/50 μg of phenylmethylsulfonyl fluoride per ml/1 μM pepstatin A/1 mM iodoacetamide. Frozen superior cervical ganglia, adrenal medullae, and sciatic nerves (Rockland Scientific) were rapidly thawed and homogenized in the sucrose homogenization buffer. Rat

skeletal muscle was dissected from the hind legs, and a light surface membrane fraction was prepared by a modification of the method of Barchi *et al.* (24). Sodium channel concentration was determined by measurement of specific binding of saxitoxin in membrane fractions at 0°C using a rapid filtration assay on GF/F filters (Whatman) at 20 nM saxitoxin (23).

Solubilization, Immunoprecipitation, and Phosphorylation of Sodium Channels. Membrane fractions were diluted to 1 nM sodium channels (100 fmol per sample) as assessed by saxitoxin binding activity in 100 mM choline chloride/10 mM EDTA/10 mM EGTA/50 mM potassium phosphate, pH 7.4/3–5% Triton X-100 containing the protease inhibitors phenylmethylsulfonyl fluoride (50 μg/ml), iodoacetamide (1 mM), and pepstatin A (1 mM). After mixing for 30 min at 4°C, the residual membranes were sedimented at 8000 × *g* for 15 min. The supernatants were incubated for 16 hr with affinity-purified antibodies at 4°C. The antigen–antibody complexes were isolated by adsorption to protein A-Sepharose (10 mg), and the pellets were washed twice with phosphorylation buffer (25). The immunoprecipitated sodium channels were radiolabeled by phosphorylation with 500 ng of cAMP-dependent protein kinase and 5 μCi (1 Ci = 37 GBq) of [γ-^{32}P]ATP for 1 min at 36°C (25).

NaDodSO$_4$ Gel Electrophoresis. Pellets from immunoprecipitation and phosphorylation were suspended in 3% NaDodSO$_4$/30 mM Tris (adjusted to pH 8.6 with HCl)/2 mM EDTA/5% sucrose/5% 2-mercaptoethanol and were boiled for 5 min. The pH was adjusted to 7.4, and the proteins were resolved by electrophoresis through a stacking gel of 3% acrylamide and a running gel with a 3–10% acrylamide gradient as described (25, 26). Radiolabeled bands were visualized by autoradiography. The intensity of autoradiographic bands was determined with a soft laser scanning densitometer (Zeineh SL-504-XL). Exposure times were selected to give a linear response of the film to the incorporated radioactivity.

RESULTS AND DISCUSSION

Specific Recognition of R_I and R_{II}. Affinity-purified antibodies were prepared against a peptide (SP1) corresponding to a conserved sequence in the R_I and R_{II} sodium channel subtypes and against two peptides ($SP11_I$ and $SP11_{II}$) corresponding to a nearby divergent sequence having only 3 widely spaced amino acids out of 18 that are common between these subtypes. Immunoprecipitation of purified, ^{32}P-labeled sodium channels by increasing concentrations of the antibodies directed against the SP1 and SP11 peptides is illustrated in Fig. 1*A*. At saturating concentrations, anti-$SP11_I$ antibodies precipitated 26% and anti-$SP11_{II}$ antibodies precipitated 70% of the ^{32}P-labeled sodium channels in this preparation that were precipitated by anti-SP1 (Fig. 1*A*). Immunoprecipitation by saturating concentrations of anti-$SP11_I$ and anti-$SP11_{II}$ was additive indicating that these antibodies immunoprecipitate different populations of purified sodium channels (Fig. 1*B*).

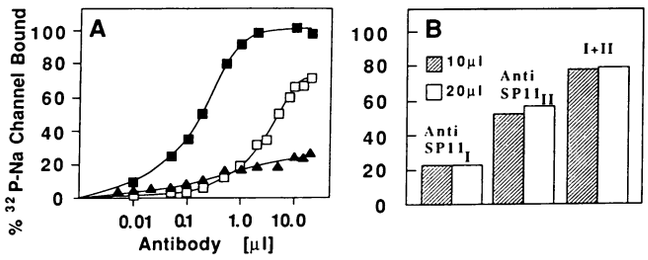

FIG. 1. Additive immunoprecipitation of the R_I and R_{II} sodium channel subtypes. (*A*) Purified, ^{32}P-labeled sodium channels (50 fmol) were immunoprecipitated with the indicated volumes of antibodies. Nonspecific immunoprecipitation (approximately 1% of total) was measured in the presence of the corresponding peptide (100 μM) and was subtracted from the data presented. ■, Anti-SP1; ▲, anti-$SP11_I$; □, anti-$SP11_{II}$. (*B*) Additivity of immunoprecipitation of R_I and R_{II} from a different purified preparation of rat brain sodium channels by saturating volumes (10 or 20 μl as indicated) of anti-$SP11_I$ and anti-$SP11_{II}$ was measured as in *A*.

The crossreactivity of the anti-$SP11_I$ and anti-$SP11_{II}$ antibodies was examined in the experiment illustrated in Fig. 2. Immunoprecipitation of ^{32}P-labeled sodium channels by anti-$SP11_I$ is reduced to 50% of maximum by 2.5 nM $SP11_I$ or 11.5 nM unlabeled sodium channels, which corresponds to approximately 2.9 nM R_I. Saturating concentrations of unlabeled sodium channels or $SP11_I$ completely block immunoprecipitation by anti-$SP11_I$, but similar concentrations of $SP11_{II}$ have no effect. Similarly, immunoprecipitation of ^{32}P-labeled sodium channels by anti-$SP11_{II}$ is reduced to 50% of maximum by 0.5 nM $SP11_{II}$ or 0.8 nM sodium channels, which corresponds to approximately 0.6 nM R_{II}. Saturating concentrations of unlabeled sodium channels or $SP11_{II}$ completely block immunoprecipitation by anti-$SP11_{II}$, but $SP11_I$ has no effect. These results show that these anti-peptide antibodies bind native sodium channels almost as well as the peptides used as antigens and that they are specific for distinct α subunit subtypes present in the purified sodium channel preparation from rat brain. It is likely that they recognize specifically the R_I and R_{II} sodium channel subtypes whose primary structures contain the corresponding amino acid sequences.

Measurement of R_I and R_{II} by Immunoprecipitation and Phosphorylation. The α subunits of rat brain sodium channels are unusually good substrates for phosphorylation by cAMP-dependent protein kinase (17), and both the R_I and R_{II} subtypes are readily phosphorylated *in vitro* (Fig. 1). The phosphorylation sites of these two sodium channel subtypes are located on the same set of tryptic phosphopeptides, indicating that R_I and R_{II} are phosphorylated at the same sites (30). Immunoprecipitating sodium channels with specific antibodies, radiolabeling the precipitated α subunits by phosphorylation with cAMP-dependent protein kinase, and analyzing by NaDodSO$_4$/PAGE provide a sensitive method for detection of sodium channels in neuronal tissues, which allows detection of 2 fmol of sodium channels in unpurified membrane extracts (25). In these experiments, we have adapted this method to analyze tissue-specific expression of the R_I and R_{II} sodium channel subtypes using sequence-specific antibodies.

Sodium channels were solubilized from a lysed crude synaptosomal membrane fraction (lysed P_3; ref. 23) from rat brain, immunoprecipitated with either anti-SP1, anti-$SP11_I$, or anti-$SP11_{II}$ antibodies, phosphorylated, and analyzed by NaDodSO$_4$/PAGE. One major phosphorylated protein band

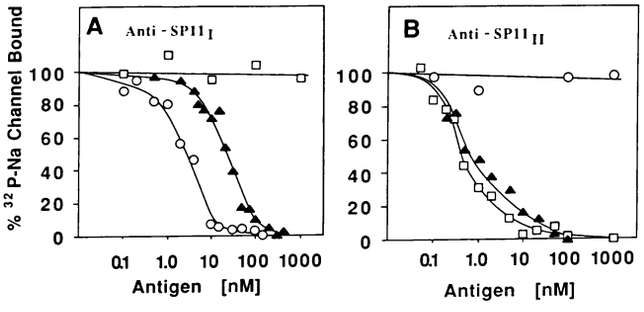

FIG. 2. Specificity of immunoprecipitation by anti-$SP11_I$ and anti-$SP11_{II}$. Fifty femtomoles of ^{32}P-labeled sodium channels were immunoprecipitated by anti-$SP11_I$ (*A*) or anti-$SP11_{II}$ (*B*) in the presence of the indicated concentrations of purified sodium channels (▲), $SP11_I$ (○), or $SP11_{II}$ (□).

of 260 kDa is observed with each antibody (Fig. 3A, lanes 1–3), but it is not observed with preimmune antiserum or with affinity-purified antibodies that have been previously blocked by incubation with the corresponding peptide antigen (data not shown). Therefore, this band represents the α subunits of the sodium channel. Anti-SP1 immunoprecipitates more labeled α subunits than either of the antibodies directed against variable sequences of the protein (Fig. 3A, lane 1). In analyzing our results, we have set the amount of radiolabeled α subunits immunoprecipitated by anti-SP1 antibodies equal to 100% (Fig. 1A) and have compared the amounts precipitated by anti-SP11$_I$ and anti-SP11$_{II}$ to that value. By this criterion, R_I and R_{II} comprise an average of 15.1% and 60.4%, respectively, of the sodium channels in membrane preparations from whole rat brain.

Several control experiments were carried out to examine whether our methods provide an accurate measurement of the ratio of expression of these two channel subtypes. Experiments with increased concentrations of antibodies confirmed that the amounts used were saturating. Measurements of the dissociation rates of the antibody–^{32}P-labeled sodium channel complex during subsequent centrifugation, phosphorylation, and washing showed that complexes with anti-SP1 and anti-SP11$_I$ antibodies are recovered quantitatively, while approximately 30% of the complexes with anti-SP11$_{II}$ antibodies are lost. We have applied a correction factor to account for this in quantitatively analyzing our results in Table 1 and Fig. 5. With this correction, our results indicate that the sodium channels in crude synaptosomal membrane preparations from whole rat brain are 15% R_I and 78% R_{II}. Comparison of these values with those for purified preparations (Fig. 1A) suggests that the R_I subtype is recovered in higher yield during purification.

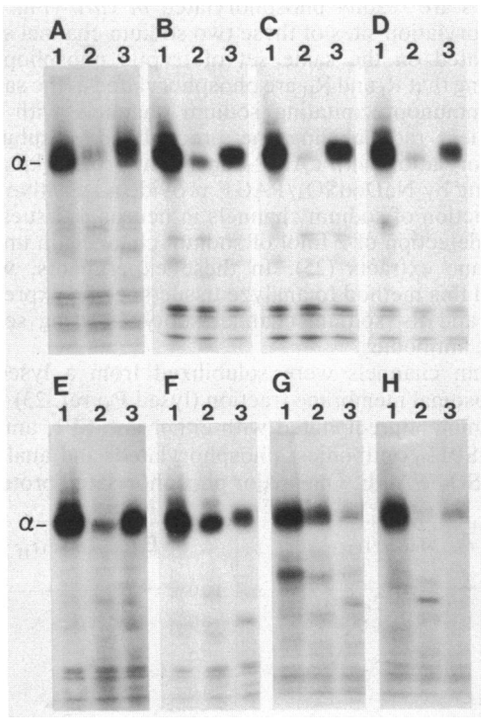

FIG. 3. Differential expression of R_I and R_{II} in the central nervous system. Membrane fractions containing 100 fmol of saxitoxin binding sites were prepared. The R_I and R_{II} sodium channels were solubilized, immunoprecipitated with anti-SP1 (lanes 1), anti-SP11$_I$ (lanes 2), or anti-SP11$_{II}$ (lanes 3) antibodies, phosphorylated, and analyzed by NaDodSO$_4$/PAGE and autoradiography. (A) Crude synaptosomal fraction (lysed P$_3$) from whole rat brain. (B) Cerebral cortex. (C) Hippocampus. (D) Midbrain. (E) Cerebellum. (F) Medulla oblongata. (G) Spinal cord. (H) Optic nerve.

Variations in the extent of endogenous phosphorylation of sodium channel subtypes or in dephosphorylation of these subtypes during tissue fractionation might influence the extent of radiolabeling of sodium channels in tissue extracts by phosphorylation. Purified R_I and R_{II} sodium channels are rapidly dephosphorylated by phosphatases in rat brain cytosolic fractions (S. Rossie and W.A.C., unpublished experiments). Prior dephosphorylation of the sodium channels in extracts of rat brain and spinal cord, which have widely differing ratios of R_I to R_{II} (see below), by incubation with a rat brain cytosol fraction before immunoprecipitation and radiolabeling had no effect on the ratios of R_I to R_{II} observed. Similarly, prior dephosphorylation of sodium channels from tissues in which these subtypes were not detected (see below) did not reveal either subtype. The extent of dephosphorylation of the cAMP-dependent phosphorylation sites on R_I and R_{II} during tissue preparation, solubilization, and incubation with antibodies was measured by the addition of purified, ^{32}P-labeled sodium channels and was found to be comparable. Thus, differential endogenous phosphorylation and differential dephosphorylation of sodium channel subtypes during tissue fractionation do not influence the results described below.

R_I and R_{II} Are Expressed Differentially in the Central Nervous System. Similar analyses of the amounts of R_I and R_{II} were carried out with tissue samples containing 100 fmol of saxitoxin binding sites from specific regions of the central nervous system. As illustrated in Fig. 3, hippocampus, cerebral cortex, and cerebellum expressed substantially less R_I than R_{II}, with R_I/R_{II} ratios ranging from 0.07 in the hippocampus to 0.17 in cerebral cortex (Table 1). In these brain regions, R_I and R_{II} accounted for greater than 90% of the sodium channels recognized by anti-SP1, the antibody we have used to define 100% in these studies. If other sodium channel subtypes are present in these brain regions, they must either comprise only a small portion of the total, lack phosphorylation sites, or fail to be recognized by these three sequence-directed antibodies and our other polyclonal antibodies. In the midbrain, R_{II} is also expressed at a higher level than R_I (Fig. 3 and Table 1). However, sodium channels that are recognized by anti-SP1 but not by anti-SP11$_I$ or anti-SP11$_{II}$ are also detected (Table 1). Nevertheless, expression of the R_{II} subtype is clearly predominant in these higher brain

Table 1. Differential expression of R_I and R_{II} in the central nervous system

Region	R_I, %	R_{II}, %	Unidentified, %	R_I/R_{II}
Whole brain				
Purified Na channels	18	81	<10	0.22
P_3 membranes	15	78	<10	0.19
Cerebral cortex	14	79	<10	0.17
Hippocampus	6	97	<10	0.07
Cerebellum	8	84	<10	0.09
Midbrain	9	56	35	0.16
Medulla oblongata	38	39	23	0.98
Spinal cord	39	18	43	2.18
Optic nerve	3	33	64	0.09
Retina	6	18	76	0.36

Autoradiograms similar to those in Fig. 3 were scanned under conditions where intensity was proportional to input protein, and the density of the α-subunit bands was quantitated. The intensity of the α band immunoprecipitated by anti-SP1 antibodies was set at 100%, and the amounts of R_I and R_{II} were estimated by comparison with that value using the correction factor described in the text. R_I/R_{II}, Ratio of R_I to R_{II}.

regions among the sodium channel subtypes that are detected by the methods used here.

In contrast, R_I was expressed at comparable or higher levels than R_{II} in medulla oblongata and spinal cord, with R_I/R_{II} ratios of 0.98 and 2.2, respectively (Fig. 3 and Table 1). In addition, in these more caudal regions of the central nervous system, unidentified sodium channel subtypes that are recognized by anti-SP1 antibodies but not by anti-SP11$_I$ or anti-SP11$_{II}$ antibodies comprised a substantial fraction of the sodium channels detected. Therefore, expression of both the R_I subtype and unidentified subtypes of sodium channels in medulla oblongata and spinal cord is increased relative to the R_{II} subtype. Since the medulla oblongata contains a substantial complement of ascending projections from neurons in the spinal cord, much of the R_I sodium channel subtype observed in the medulla could be synthesized by spinal neurons.

The optic nerve and retina are also considered projections of the central nervous system. As in the brain, R_{II} was expressed preferentially compared to R_I in the optic nerve and retina, with R_I/R_{II} expression ratios of 0.09 and 0.36, respectively (Fig. 3 and Table 1). However, in contrast to other regions of the central nervous system, the unidentified sodium channel subtype(s) was the predominant form expressed in optic nerve and retina and accounts for 64% and 76% of the sodium channels detected, respectively.

R_I and R_{II} Are Expressed Primarily in the Central Nervous System. In previous studies, we have shown by radioimmunoassay that sodium channels in the peripheral nervous system and skeletal muscle are poorly recognized by polyclonal antibodies against rat brain sodium channels (12). To extend these studies, we first examined if the sodium channels in several peripheral excitable tissues were recognized by the anti-SP1 antibodies, which are directed against a conserved epitope common to R_I, R_{II}, and additional unidentified sodium channels subtype(s) expressed in the central nervous system. These sodium channel subtypes were not detected by immunoprecipitation and phosphorylation with anti-SP1 antibodies in samples containing 100 fmol of saxitoxin binding sites from skeletal muscle (Fig. 4) or heart (data not shown), suggesting that R_I and R_{II} are expressed primarily in the nervous system. Similarly, these channel subtypes were also not detected in sympathetic ganglia or adrenal medulla (Fig. 4), suggesting that R_I and R_{II} are not expressed by neurons of the autonomic nervous system or by endocrine cells. Moreover, neither R_I, R_{II}, nor the additional unidentified sodium channel subtypes recognized by anti-SP1 antibodies in the central nervous system were detected in sciatic nerve, which contains the peripheral projections of spinal motor neurons, or in cauda equina, the most caudal segment of the spinal cord, which contains fiber tracts within the lower vertebrae (Fig. 4). These results contrast with expression of these subtypes in the central myelinated fibers of the optic nerve (Fig. 3) and corpus callosum (data not shown). Thus, previous results (12) and the work presented here suggest that these channel subtypes are expressed primarily, if not exclusively, by neurons in the central nervous system and are excluded from the peripheral projections of central neurons.

Since expression of the R_I and R_{II} sodium channel subtypes is restricted to the central nervous system, it was of interest to examine if the antigenic epitopes that we have used to define these subtypes are conserved in the central nervous systems of a range of species. Sodium channels that are recognized by anti-SP1 antibodies were detected in mammalian (rat and monkey), avian (chicken), reptilian (gecko), and amphibian (frog) brains but not in a bony fish (electric eel) brain (Fig. 4*B*). However, sodium channels that are recognized by anti-SP11$_I$ or anti-SP11$_{II}$ were observed only in monkey and rat brain. Although only a few species have been examined, it appears that these epitopes may be conserved among mammals but are not conserved in most nonmammalian vertebrates.

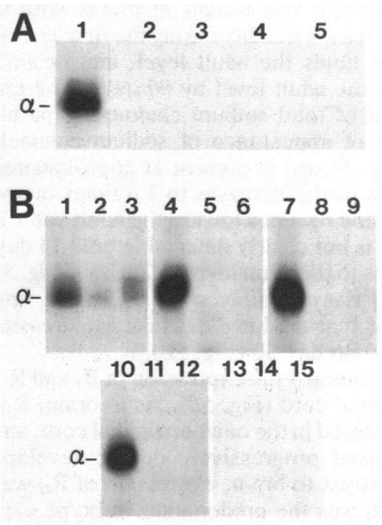

FIG. 4. Expression of R_I and R_{II} in excitable tissues. (*A*) Membrane fractions containing 100 fmol of saxitoxin binding sites were prepared from rat brain (lane 1), superior cervical ganglia (lane 2), skeletal muscle (lane 3), sciatic nerve (lane 4), and adrenal medulla (lane 5); sodium channels were solubilized, immunoprecipitated with anti-SP1 antibodies, phosphorylated, and analyzed by $NaDodSO_4$/PAGE and autoradiography. (*B*) Membrane fractions were prepared from brains of the indicated species, and sodium channels were solubilized, immunoprecipitated with anti-SP1 (lanes 1, 4, 7, 10, and 13), anti-SP11$_I$ (lanes 2, 5, 8, 11, and 14), or anti-SP11$_{II}$ (lanes 3, 6, 9, 12, and 15), and analyzed by $NaDodSO_4$/PAGE and autoradiography. Lanes: 1–3, monkey (*Macaca nemestrina*) brain; 4–6, chicken (*Gallus gallus*) brain; 7–9, gecko (*Gecko gecko*) brain; 10–12, frog (*Rana pipiens*) brain; 13–15, eel (*Electrophorus electricus*) brain.

Expression of R_I and R_{II} Is Differentially Regulated During Development. Analysis of the relative expression of R_I and R_{II} in brain and spinal cord during development reveals additional levels of regulation (Fig. 5). At birth, the number of sodium channels in rat or mouse brain per unit wet weight, as measured by high affinity binding of saxitoxin, is less than 10% of the adult level (27–29). The density of sodium

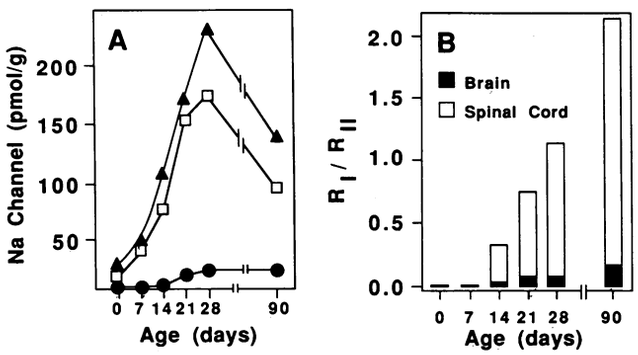

FIG. 5. Developmental regulation of the expression of R_I and R_{II}. (*A*) Brains were rapidly dissected from rats of the indicated ages, the cerebella were removed, and membrane fractions were isolated. Specific saxitoxin binding (▲) was measured at a saturating concentration (20 nM). Sodium channels were solubilized, immunoprecipitated with anti-SP11$_{II}$ (□) or anti-SP11$_I$ (●) antibodies, phosphorylated, and analyzed by $NaDodSO_4$/PAGE and autoradiography. The percentages of R_I and R_{II} were multiplied by the total number of saxitoxin binding sites to give pmol of R_I and R_{II} per mg of protein. (*B*) A similar experiment was carried out with rat spinal cord, and the data for both brain and spinal cord were plotted as the ratio of R_I/R_{II}.

channels per unit wet weight in brains with the cerebella removed increases steadily during the first 28 days after birth, reaching 1.8 times the adult level, and declines thereafter returning to the adult level by 90 days. The time course of development of total sodium channels is paralleled by the time course of appearance of sodium channels of the R_{II} subtype (Fig. 5). R_{II} is present at approximately 7% of the adult level at birth, increases to 1.8 times the adult level by days 21–28, and declines toward the adult value by day 90. In contrast, R_I is not clearly detectable until 14 days after birth and increases to the adult level by 28 days (Fig. 5*A*). The ratio of R_I to R_{II} rises steadily over the entire time course of development that we have examined, increasing from 0 on day 7 to 0.19 on day 90 (Fig. 5*B*).

We also examined the expression of R_I and R_{II} in newborn and adult spinal cord (Fig. 5*B*). As in brain, R_{II} was preferentially expressed in the newborn spinal cord, and expression of R_I increased progressively during development in the adult. In contrast to brain, expression of R_{II} was reduced in adult, and R_I was the predominant subtype expressed.

Tissue-Specific Expression of Sodium Channel Subtypes. Three or more different sodium channel subtypes are expressed in the rat central nervous system: R_I, R_{II}, and at least one unidentified subtype that is recognized by anti-SP1 antibodies but not by anti-$SP11_I$ or anti-$SP11_{II}$ antibodies. The unidentified subtype that we have detected in the central nervous system in our immunoprecipitation experiments may be encoded by the third sodium channel mRNA that Noda *et al.* (8) detected in rat brain cDNA libraries but did not sequence fully. R_I is preferentially expressed in the spinal cord, R_{II} is preferentially expressed in the brain, and the unidentified subtype(s) recognized by anti-SP_I antibodies is preferentially expressed in retina and optic nerve and to a lesser extent in the spinal cord. In addition to these three or more sodium channel subtypes in the central nervous system, our results provide clear evidence that the sodium channels that are expressed in the peripheral nervous system represent one or more distinct subtypes. Polyclonal antibodies against rat brain sodium channels and anti-peptide antibodies directed against the SP1, $SP11_I$, and $SP11_{II}$ segments of the α subunit all recognize sodium channels in the central nervous system but not those in the peripheral nervous system (ref. 12 and this paper). Sodium channel subtypes other than R_I and R_{II} must also be expressed in skeletal and cardiac muscle because our experiments with specific antibodies do not detect R_I or R_{II} in muscle tissues (ref. 12 and Fig. 4). Correlation of differences in pharmacological and physiological properties with differences in the primary structure of these six or more sodium channel subtypes is an important area for future work.

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1. Agnew, W. (1984) *Annu. Rev. Physiol.* **46,** 517–530.
2. Barchi, R. L., Tanaka, J. D. & Furman, R. E. (1984) *J. Cell. Biochem.* **26,** 135–146.
3. Catterall, W. A. (1984) *Science* **223,** 653–661.
4. Catterall, W. A. (1986) *Annu. Rev. Biochem.* **55,** 953–985.
5. Noda, M., Shimizu, S., Tanabe, T., Takai, T., Kayano, T., Ikeda, T., Takahashi, H., Nakayama, H., Kanaoka, Y., Minamino, N., Kangawa, K., Matsuo, H., Raftery, M., Hirose, T., Inayama, S., Hayashida, H., Miyata, T. & Numa, S. (1984) *Nature (London)* **312,** 121–127.
6. Auld, V., Marshall, J., Goldin, A., Dowsett, A., Catterall, W., Davidson, N. & Dunn, R. (1985) *J. Gen. Physiol.* **86,** 10a (abstr.).
7. Mandell, G., Cooperman, S., Montminy, M., Barchi, R., Brehm, P. & Goodman, R. (1986) *Biophys. J.* **49,** 380a (abstr.).
8. Noda, M., Ikeda, T., Kayano, T., Suzuki, H., Takeshima, H., Kurasaki, M., Takahashi, H. & Numa, S. (1986) *Nature (London)* **320,** 188–192.
9. Goldin, A. L., Snutch, T., Lübbert, H., Dowsett, A., Marshall, J., Auld, V., Downey, W., Fritz, L., Lester, H., Dunn, R., Catterall, W. & Davidson, N. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 7503–7507.
10. Sumikawa, K., Parker, I. & Miledi, R. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 7994–7998.
11. Noda, M., Ikeda, T., Kayano, T., Suzuki, H., Takeshima, H., Kurasaki, M., Takahashi, H. & Numa, S. (1986) *Nature (London)* **320,** 188–192.
12. Wollner, D. & Catterall, W. A. (1985) *Brain Res.* **331,** 145–149.
13. Beavo, J. A., Bechtel, P. J. & Krebs, E. G. (1974) *Methods Enzymol.* **38,** 299–309.
14. Ritchie, J. M., Rogart, R. B. & Strichartz, G. R. (1976) *J. Physiol. (London)* **261,** 477–494.
15. Waechter, C. J., Schmidt, J. & Catterall, W. A. (1983) *J. Biol. Chem.* **258,** 5117–5123.
16. Hartshorne, R. & Catterall, W. A. (1984) *J. Biol. Chem.* **259,** 1667–1675.
17. Costa, M. & Catterall, W. A. (1984) *J. Biol. Chem.* **259,** 8210–8218.
18. Merrifield, R. B. (1963) *J. Am. Chem. Soc.* **85,** 2149–2154.
19. Hunter, W. M. & Greenwood, E. C. (1962) *Nature (London)* **194,** 495–496.
20. Bolton, A. E. & Hunter, W. M. (1973) *Biochem. J.* **133,** 529–538.
21. Orth, D. N. (1979) in *Methods of Hormone Radioimmunoassay*, eds. Jaffe, B. M. & Behrman, H. R. (Academic, New York), pp. 245–284.
22. Olmsted, J. B. (1981) *J. Biol. Chem.* **256,** 11955–11957.
23. Catterall, W. A., Morrow, C. & Hartshorne, R. (1979) *J. Biol. Chem.* **254,** 11379–11387.
24. Barchi, R. L., Weigele, J. B., Chalikian, D. M. & Murphy, L. E. (1979) *Biochim. Biophys. Acta* **550,** 59–76.
25. Schmidt, J., Rossie, S. & Catterall, W. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 4847–4851.
26. Maizel, J. V. (1971) *Methods Virol.* **51,** 179–224.
27. Unsworth, B. & Hafemann, D. (1975) *J. Neurochem.* **24,** 261–270.
28. Baumgold, J., Zimmerman, I. & Bambrick, L. (1983) *Dev. Brain Res.* **9,** 405–407.
29. Lombet, A., Kazazoglou, T., Delpont, E., Renaud, J. & Lazdunski, M. (1984) *Biochem. Biophys. Res. Commun.* **110,** 894–901.
30. Rossie, S., Gordon, D. & Catterall, W. A. (1987) *J. Biol. Chem.*, in press.